MLVA 16

1. PCR amplification

1) Sample DNA

Use 2μl of DNA extract (5ng/μl) per amplification reaction.

2) Primers (16)

	Primer	Sequence (5'-3')
1	ETRA-2165_75bp_397bp_3U	L)ATT TCG ATC GGG ATG TTG AT R)TCG GTC CCA TCA CCT TCT TA
2	ETRB-2461_57bp_292bp_3U	L)GCG AAC ACC AGG ACA GCA TCA TG R)GGC ATG CCG GTG ATC GAG TGG
3	ETRC-0577_58bp_346bp_4U	L)GAC TTC AAT GCG TTG TTG GA R)GTC TTG ACC TCC ACG AGT GC
4	ETRD-0580_77bp_330bp_3U	L)GCG CGA GAG CCC GAA CTG C R)GCG CAG CAG AAA CGT CAG C
5	ETRE-3192_53bp_651bp_3U	L)ACT GAT TGG CTT CAT ACG GCT TTA R)GTG CCG ACG TGG TCT TGA T
6	MIRU10-0959_53bp_643bp_3U	L)GTT CTT GAC CAA CTG AGT CGT CC R)GCC ACC TTG GTG ATC AGC TAC CT
7	MIRU16-1644_53bp_671bp_2U	L)TCG GTG ATC GGG TCC AGT CCA AGT A R)CCC GTC GTG CAG CCC TGG TAC
8	MIRU23-2531_53bp_873bp_6U	L)CAG CGA AAC GAA CTG TGC TAT CAC R)CGT GTC CGA GCA GAA AAG GGT AT
9	MIRU26-2996_51bp_613bp_3U	L)CCC GCC TTC GAA ACG TCG CT R)TGG ACA TAG GCG ACC AGG CGA ATA
10	MIRU27-3006_53bp_657bp_3U	L)TCG AAA GCC TCT GCG TGC CAG TAA R)GCG ATG TGA GCG TGC CAC TCA A
11	MIRU39-4348_53bp_646bp_2U	L)CGC ATC GAC AAA CTG GAG CCA AAC R)CGG AAA CGT CTA CGC CCC ACA CAT
12	MIRU40-0802_54bp_407bp_1U	L) GGG TTG CTG GAT GAC AAC GTG T R) GGG TGA TCT CGG CGA AAT CAG ATA
13	Mtub02-0079_9bp_230bp_6U	L) CGT GCA CAG TTG GGT GTT TA R) TTC GTT CAG GAA CTC CAA GG
14	Mtub21-1955_57bp_206bp_2U	L)AGA TCC CAG TTG TCG TCG TC R)CAA CAT CGC CTG GTT CTG TA
15	Mtub30-2401_58bp_319bp_2U	L)AGT CAC CTT TCC TAC CAC TCG TAA C R)ATT AGT AGG GCA CTA GCA CCT CAA G
16	Mtub39-3690_58bp_515bp_6U	L) AAT CAC GGT AAC TTG GGT TGT TT R) GAT GCA TGT TCG ACC CGT AG

3) Preparation of working master mix.

(1) Preparation for the working master mix for 100 reactions

Reagent	Volume(μl)	*100Rns(μl)
10XBuffer(Including $MgCl_2$)	1.5	150
dNTP	1.5	150
5MBetain	3.0	300
Primers 100μM(Left and Right mixture)	1.5	100
Taq E (5U/μl)	0.05	5
Distilled Water	5.5	600
Total	13.0	1300

(2) Dispense 13μl of working master mix to each PCR reaction tube.

(3) Add 2μl of DNA samples(5ng/μl) to each reaction vial as given below.

4) Thermal cycling protocol

The cycling protocol is as follows:

94℃ 5min

94℃ 30sec

62℃ 30sec 35cycles

72℃ 45sec

72℃ 10min

20℃ until the gel is loaded for electrophoresis

2. Detection and Analysis of PCR Products by Agarose Gel Electrophoresis(AGE)

1. Agarose gel electrophoresis

1) Prepare a 2.0% (3.0% for Mtub02) agarose gel using 0.5XTBE buffer (the repeat of Mtub02 is 9bp).

450ml Agarose Gel is appropriate for one large Gel tank.

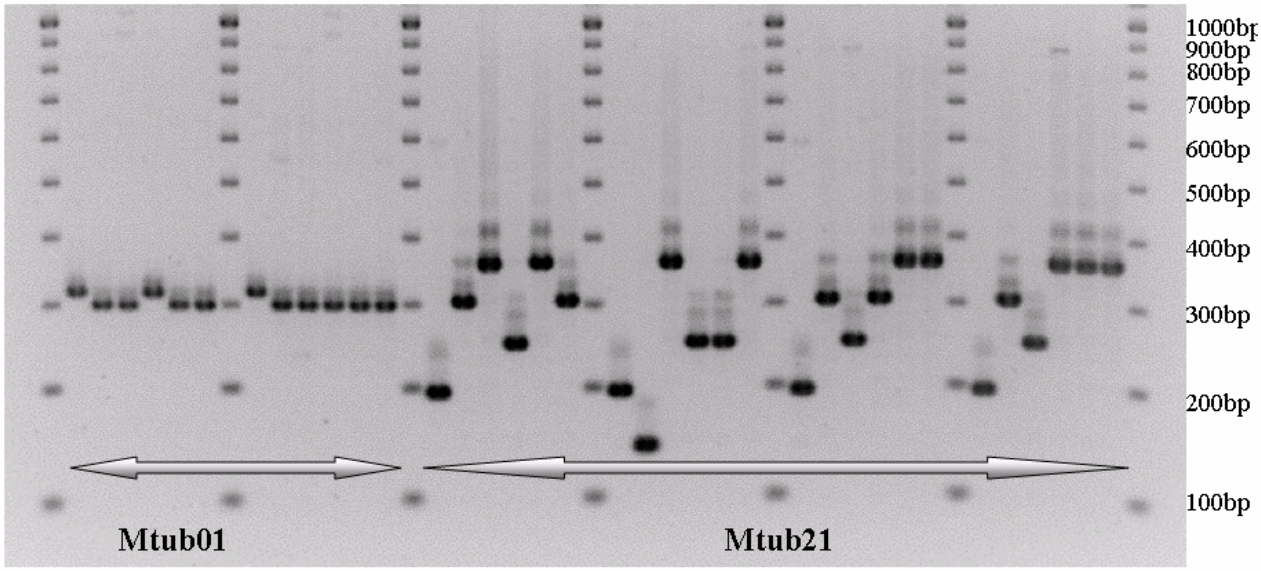
2) Add 3μl of loading dye (5X) to 15μl of PCR product, mix.

3) Load 2.5μl 100bp-lader DNA marker and 3.0μl PCR production into the gel.

4) Run at 8volt/cm till the purple dye reach the end of the gel.

5)Stain with ethidium bromide for 30min.

5) View bands on the UV trans-illuminator and take the photograph.



Data storage and analysis

The BioNumerics software is currently used to store information on strain and patients, images and genotyping data.

http://bacterial-genotyping.igmors.u-psud.fr/